Supporting Information

Tertiary Contact Formation in α-Synuclein Probed by Electron Transfer

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Analysis of ^{1,3}W* Decay Kinetics:

The probability distribution describing the distance between the ends of a freely jointed chain is:[1]

$$P_{FJC}(r)\,dr = \left(\frac{3}{2\pi\langle r^2\rangle}\right)^{3/2} \exp\left(\frac{3r^2}{2\langle r^2\rangle}\right) 4\pi r^2\,dr$$

where $\langle r^2\rangle$ is the mean squared donor acceptor distance.
The probability distribution describing the distance between the ends a worm-like chain is:[2]

$$P_{WLC}(r)\,dr = \frac{N}{l_c^2\left[1-(r/l_c)^2\right]^{5/2}} \exp\left(-\frac{3l_c}{4l_p\left[1-(r/l_c)^2\right]}\right) 4\pi r^2\,dr$$

where l_c is the contour length of the polypeptide, l_p is the persistence length of the chain segments, and N is a normalization constant. The contour length is usually taken to be $b_0 l$, where l is the number of peptide bonds in the chain, and b_0 is distance between adjacent C_α atoms.

The modified Smoluchowski equations describing intrachain diffusion of the polypeptide with energy-transfer quenching of ^{1}W*, and electron-transfer quenching of ^{3}W* are given by:

$$\frac{\partial}{\partial t}\,{}^1P^*(r,t) = -\left[{}^1k_0 + {}^1k(r)\right]{}^1P^*(r,t) + \frac{1}{r^2}\frac{\partial}{\partial r}Dr^2\,\frac{\partial}{\partial r}\,{}^1P^*(r,t) + \frac{1}{r^2}\frac{\partial}{\partial r}D\left(r^2\,{}^1P^*(r,t)\frac{\partial}{\partial r}\beta U(r)\right)$$

$$\frac{\partial}{\partial t}\,{}^3P^*(r,t) = {}^1k_0\,{}^1P^*(r,t) - \left[{}^3k_0 + {}^3k(r)\right]{}^3P^*(r,t) + \frac{1}{r^2}\frac{\partial}{\partial r}Dr^2\,\frac{\partial}{\partial r}\,{}^3P^*(r,t) + \frac{1}{r^2}\frac{\partial}{\partial r}D\left(r^2\,{}^3P^*(r,t)\frac{\partial}{\partial r}\beta U(r)\right)$$

where $^1k_0$ and $^3k_0$ are the decay rate constants for ^{1}W* and ^{3}W* in the absence of quenching; $^1k(r)$ and $^3k(r)$ are the distance-dependent quenching rate constants for for ^{1}W* and ^{3}W*; $^1P^*(r,t)$ and $^3P^*(r,t)$ represent the probabilities of finding an ^{1}W* and ^{3}W* with a quencher at distance r at time t after excitation; D is the intrachain diffusion coefficient which we have taken to be independent of r, and $\beta = 1/k_BT$. We have implicitly assumed unit efficiency for $^1\text{W}^* \rightarrow {}^3\text{W}^*$ intersystem crossing in the absence of quencher.

The potential of mean force ($U(r)$) is related to the equilibrium distribution:

$$P(r) = \exp\left[-\beta U(r)\right]$$

The distance dependent rate constants are given by Förster theory and semiclassical electron transfer theory:

$$^1k(r) = {}^1k_0\left(\frac{r_0}{r}\right)^6 + 10^{13}\exp[-1.5(r-r_c)]; \qquad ^3k(r) = 10^{13}\exp[-1.5(r-r_c)]$$

where r_0 is the Förster distance (~26 Å for the ^{1}W*/Y(NO_2) pair), and r_c is the van der Waals contact distance (~3 Å).

At t = 0, the ^{1}W* distribution will be described by the equilibrium distribution:

$$^1P^*(r,0) = P(r);$$

and the triplet will be unpopulated:

$$^3P^*(r,0) = 0$$

These coupled partial differential (Smoluchowski) equations are solved numerically using the *PDEPE* function in MATLAB (The Mathworks, Inc.), subject to the foregoing initial condition and reflective boundary conditions, to give $^1P^*(r,t)$ and $^3P^*(r,t)$.

^{1}W* and ^{3}W* decay kinetics for the full ensemble of polypeptides will be given by integrating over r:

$$^1I(t) \propto \int_{r_c}^{\infty} {}^1P^*(r,t)\,dr; \qquad ^3I(t) \propto \int_{r_c}^{\infty} {}^3P^*(r,t)\,dr$$

Calculated decays were fit to experimental $^1I(t)$ and $^3I(t)$ by adjusting the equilibrium distribution ($P(r)$) and the diffusion coefficient (D). With the FJC distribution, the adjustable parameter was $\langle r^2 \rangle$. With the WLC model, l_p was the parameter. In model-free fits, a discrete distribution was used with a 2-Å spacing between components.

The nonexponential ^{1}W* decay kinetics commonly found in W-containing peptides introduce an additional complication. The multiple ^{1}W* exponential decay components are usually attributed to multiple conformers of the indole sidechain in W. This local heterogeneity is assumed to not affect the $^1P^*(r,t)$ distributions. Hence, each W decay component is assumed to contribute independently to the total $^1P^*(r,t)$, weighted by its contribution to the unquenched ^{1}W* decay.

(1) Zhou, H.-X. *Biochemistry* **2004**, *43*, 2141-2154.
(2) Schuler, B.; Lipman, E. A.; Steinbach, P. J.; Kumke, M.; Eaton, W. A. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 2754-2759.

Table S1

	W4-Y19	Y19-W39	Y74-W94	W4-Y39	W94-Y136	W4-Y136
n^a	15	20	20	35	42	132
$A_1{}^b$	0.79	0.73	0.76	0.89	0.81	0.88
k_1 (s^{-1})^b	7.6×10^6	2.4×10^6	2.8×10^6	1.6×10^6	1.2×10^6	8.4×10^5
$A_2{}^b$	0.21	0.27	0.24	0.11	0.19	0.12
k_2 (s^{-1})^b	1.0×10^5	1.3×10^4	4.3×10^4	8.4×10^4	1.2×10^5	7.9×10^4
$\langle r \rangle_{FJC}$ (Å)^c	25	29	31	40	40	54
$\langle r^2 \rangle_{FJC}$ (Å^2)^d	720	960	1100	1910	1910	3370
D ($cm^2\ s^{-1}$)^e	2.0×10^{-6}	1.8×10^{-6}	3.4×10^{-6}	1.1×10^{-5}	6.3×10^{-6}	2.9×10^{-5}
$\langle r \rangle_{WLC}$ (Å)^c	23	27	29	38	39	58
$\langle r^2 \rangle_{WLC}$ (Å^2)^d	610	820	920	1680	1750	3880
$l_p{}^f$ (Å)	5.88	5.77	6.52	6.56	5.64	3.89
D ($cm^2\ s^{-1}$)^e	1.8×10^{-6}	1.6×10^{-6}	3.0×10^{-6}	9.5×10^{-6}	5.9×10^{-6}	5.2×10^{-5}
$\langle r \rangle_{MI}$ (Å)^c	25	31	38	47	36	53
$\langle r^2 \rangle_{MI}$ (Å^2)^d	840	1290	1880	2860	1610	3660
D ($cm^2\ s^{-1}$)^e	3.0×10^{-6}	3.2×10^{-6}	9.5×10^{-6}	6.0×10^{-6}	1.6×10^{-6}	5.0×10^{-6}

[a]number of residues separating W and $Y(NO_2)$

[b]amplitudes (A) and rate constants (k) extracted from biexponential fits ($I(t) = c + A_1\exp(-k_1 t) + A_2\exp(-k_2 t)$) of $^3W^*$ decays

[c]mean distance $\langle r \rangle$ extracted from respective distribution functions: FJC (freely jointed chain), WLC (wormlike chain), and MI (model independent); calculated using numerical integration ($\langle r \rangle = \int rP(r)\,dr / \int P(r)\,dr$) for continuous distributions (FJC and WLC) and summation ($\langle r \rangle = \sum r\mathrm{P(r)} / \sum \mathrm{P(r)}$)for discrete distribution (MI) .

[d]mean squared distance $\langle r^2 \rangle$ extracted from respective distribution functions: FJC (freely jointed chain), WLC (wormlike chain), and MI (model independent); calculated using numerical integration ($\langle r^2 \rangle = \int r^2 P(r)\,dr / \int P(r)\,dr$) for continuous distributions (FJC and WLC) and summation ($\langle r^2 \rangle = \sum r^2\mathrm{P(r)} / \sum \mathrm{P(r)}$)for discrete distribution (MI)

[e]Diffusion coefficients D extracted from simultaneously fitting to $^1W^*$ and $^3W^*$ decays to respective distribution functions: FJC (freely jointed chain), WLC (wormlike chain), and MI (model independent)

[f]persistence length of the chain segment extracted from the wormlike chain model

Figure 1S

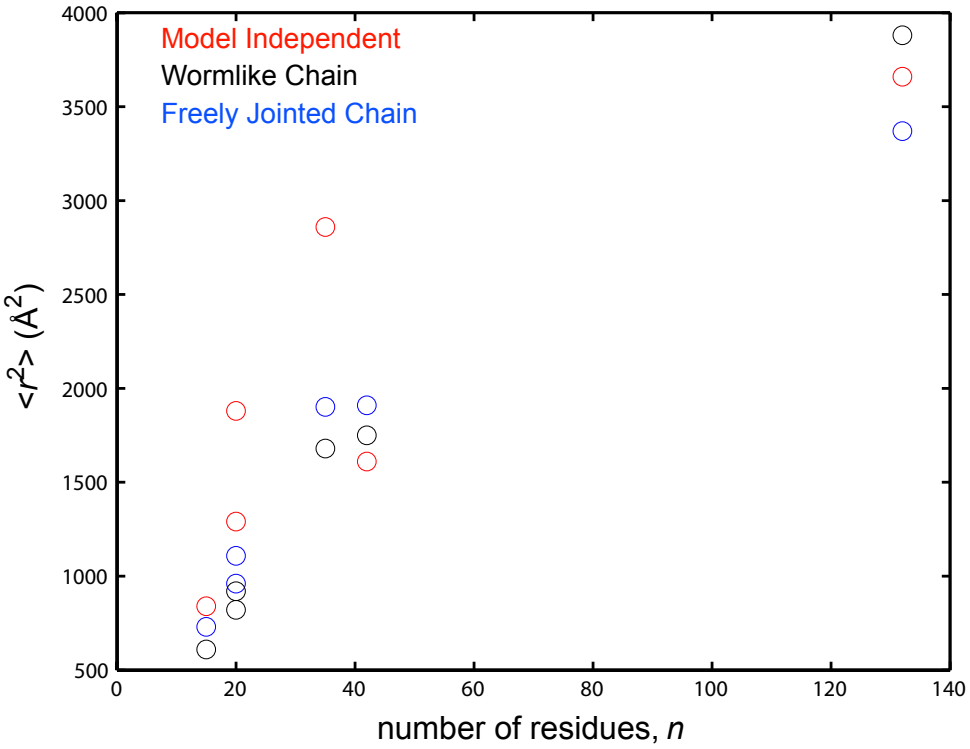


Figure 1S. Dependence of mean squared distance $\langle r^2 \rangle$ extracted from respective models on the number of residues, n, separating W and Y(NO_2).

Figure 2S

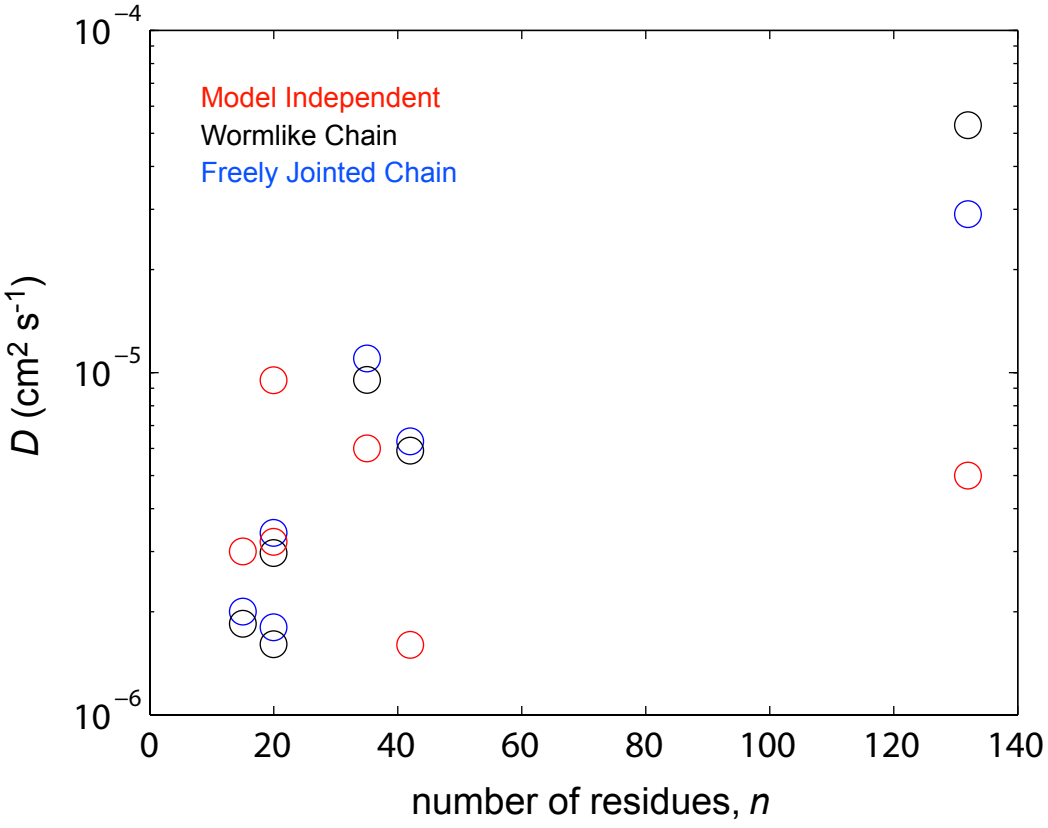
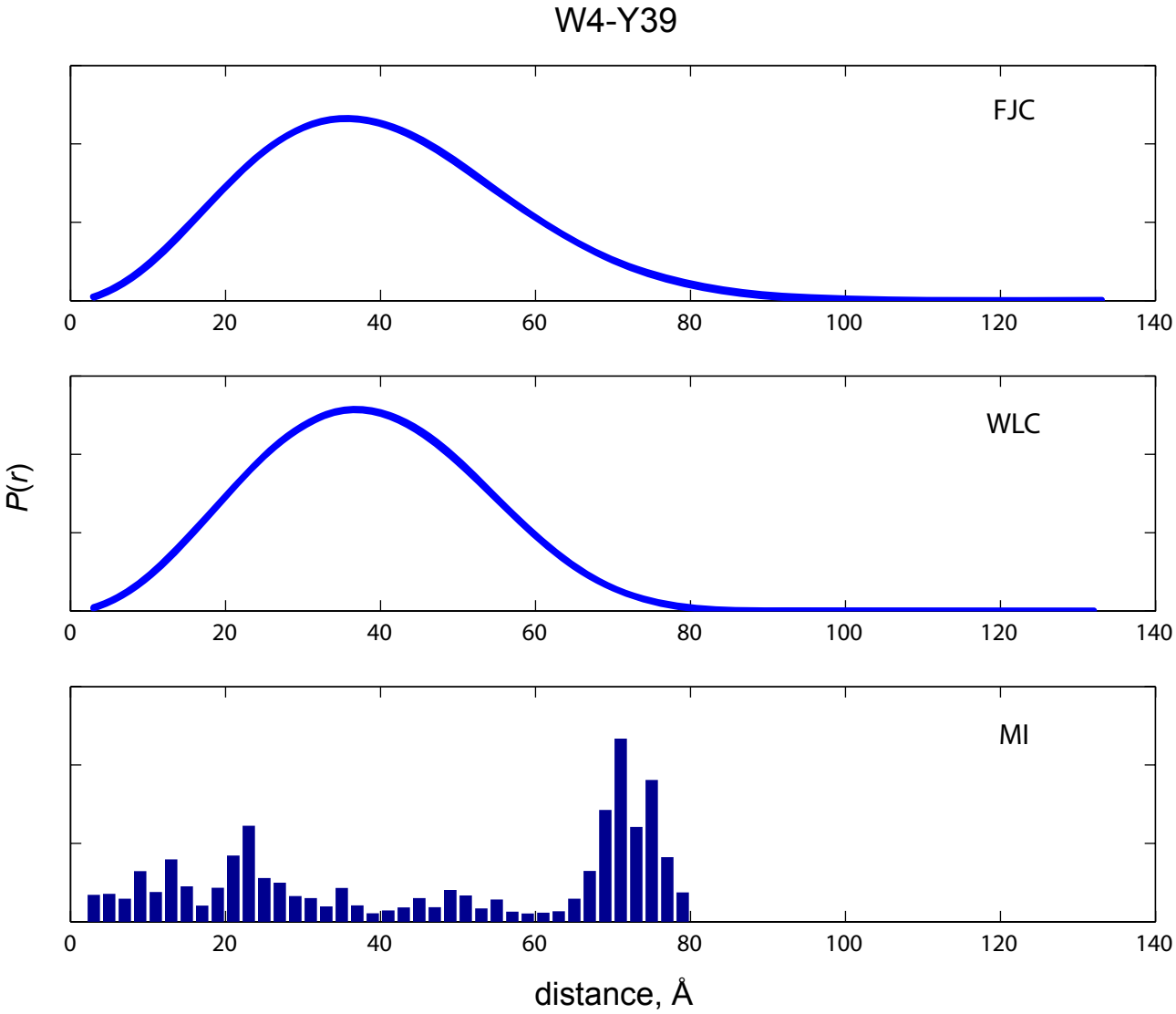
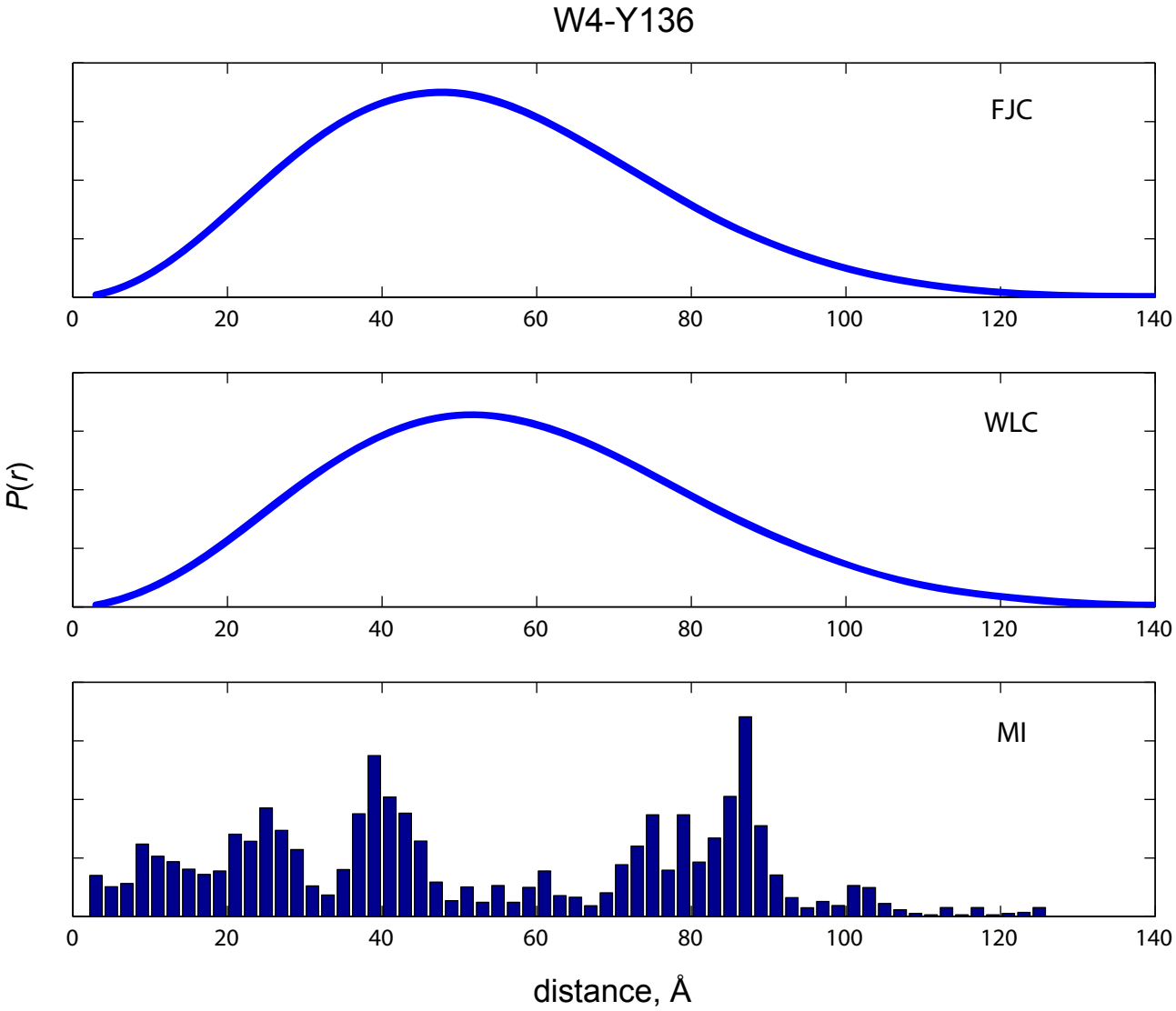
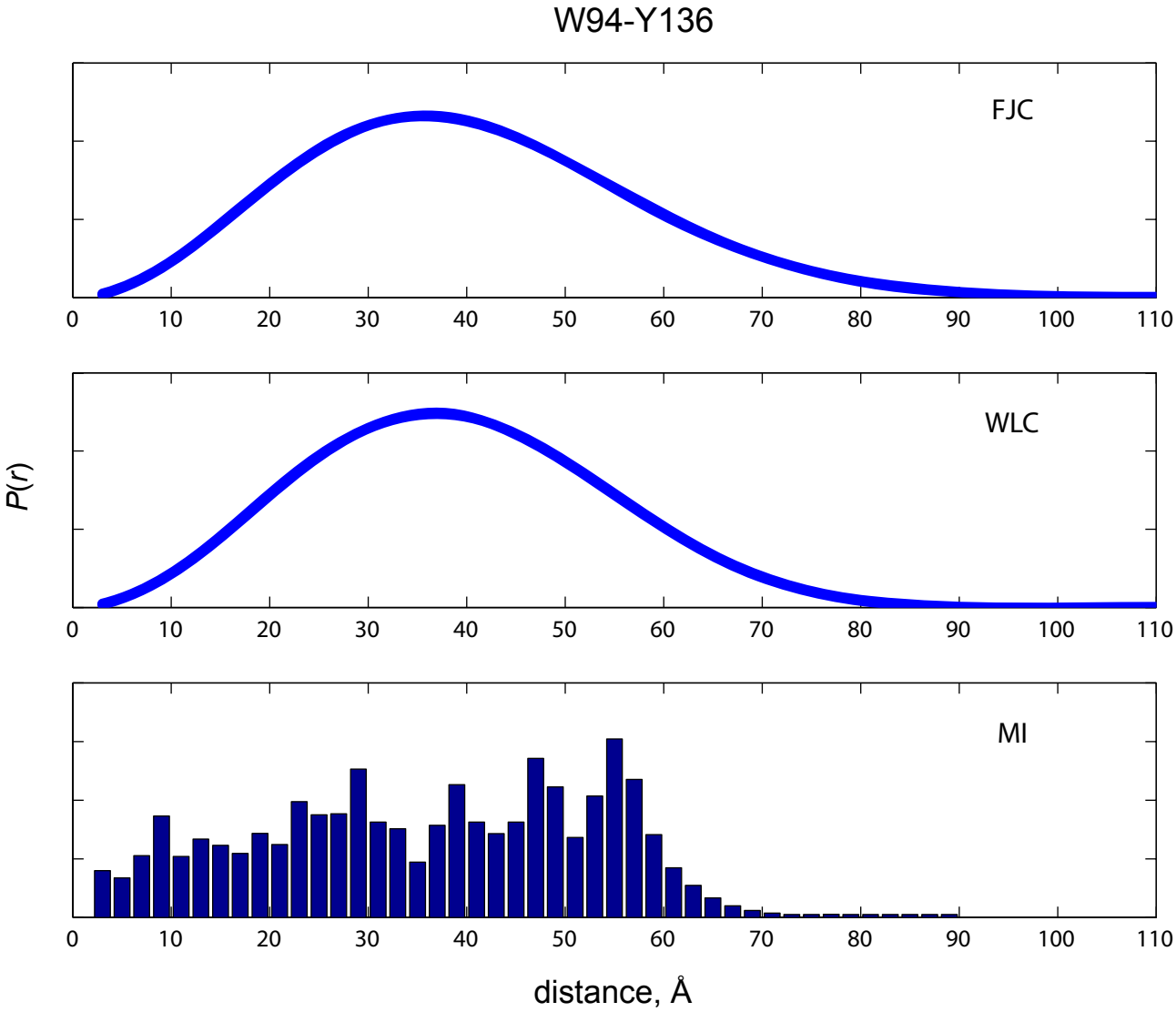


Figure 2S. Dependence of diffusion coefficient D extracted from respective models on the number of residues, n, separating W and Y(NO_2).


W4-Y39
FJC
WLC
MI
P(r)
0
20
40
60
80
100
120
140
distance, Å


W4-Y136
FJC
WLC
MI
P(r)
0
20
40
60
80
100
120
140
distance, Å


W94-Y136
FJC
WLC
MI
P(r)
0
10
20
30
40
50
60
70
80
90
100
110
distance, Å